EVALUATION OF NEW ANTIPARASITIC DRUGS AND VACCINES IN THE TROPICS

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1. Evaluation of *Plasmodium cynomolgi* Sporozoite Induced Infections of Captive Born *Macaca fasicularis*

PROBLEM : India has ceased exportation of rhesus monkeys which are used in the *Plasmodium cynomolgi* antimalarial compound testing model. A systematic evaluation of captive born *Macaca fasicularis* has not been completed to determine if this species could be used to supplement scarce rhesus monkeys.

PROGRESS : Twenty-five AFRIMS produced cynomolgus have been inoculated with *P. cynomolgi* sporozoites. The intact cynomolgus does not develop an infection comparable to the rhesus. The splenectomized captive-born cynomolgus monkey appears to be capable of supplementing rhesus in antimalarial compound testing. Relapse occurs (as in rhesus monkeys) after clearance of blood froms with chloroquine given alone or along with a noncurative dose of primaquine or test compound.

FUTURE OBJECTIVES :

1. Gather data on a larger number of splenectomized cynomolgus for significant comparison to the rhesus.

2. Use this model for evaluating potentially toxic compounds.

2. Evaluation of Experimental Antimalarial Drugs for Radical Curative Activity in the Rhesus Monkeys

PROBLEM : The ability of the malarial organism to become resistant to standard therapeutic agents is well known. Primaquine is the primary radical curative agent at present and its use is accompanied by several adverse effects. This has prompted a search for equally or more effective agents which are less toxic than primaquine.

OBJECTIVES : To test candiate antimalarial compounds for radical curative activity in the sporozoite induced *Plasmodium cynomolgi* - Rhesus model.

PROGRESS : The mosquito-monkey *P. cynomolgi* cycle has been reestablished and 45 rhesus and splenectomized cynomolgus monkeys have been used in the antimalarial drug development program during FY 83. A primaquine baseline study has been completed. Thirteen new compounds have been received for testing. Radical curative screening has been completed on 7 compounds and 25 compounds are currently in progress. Thirty young U.S. origin female rhesus and 17 adult malaria virgin rhesus females from USAMRIID were shipped to AFRIMS for compound screening in FY 83. In FY 84, AFRIMS produced young rhesus will be used for radical curative testing. Potentially toxic compounds are being screened in splenectomized cynomolgus also produced in the AFRIMS breeding colony.

FUTURE OBJECTIVES : To continue screening compounds and attempt to decrease present backlog of untested compounds.

3. Leptospirosis in the Hamster : Chemoprophylaxis in the Acute Infection

PROBLEM : Leptospirosis is a worldwide zoonotic illness that is common in tropical areas and recently has been a cause of outbreaks of acute "flu-like" illness in troops in jungle training exercises. Presently the non-human primate is being studied in our laboratory as a model for acute leptospirosis and chemophrophylaxis. A hamster model has been previously described for acute leptospirosis. A clinical, often fatal illness results in hamsters infected with some strains of leptospira. This animal model will be a useful addition to the primate model for testing potential prophylactic drug treatment for leptospirosis. Also the hamster is an ideal laboratory animal for isolating strains of leptospira from samples contaminated with other bacterial or fungal organisms. Proposed studies on the epidemiology of leptospirosis will necessitate the use of hamsters and familiarity with this model for isolation of leptospira from infected water sources.

OBJECTIVES :

1. To characterize the acute *Leptospira bataviae* infection in the hamster and determine the virulence and infectivity of the organism.

2. To determine the benefit of doxycycline prophylactic treatment in leptospira infected hamsters.

3. To obtain serovar specific antisera for developing an ELISA method to detect the acute infection.

PROGRESS : *Leptospira bataviae* isolates from patients in Thailand have been shown to be virulent and produce a chronic renal infection in hamsters with intraperitoneal doses containing as few as 1-10 organisms. A single oral dose of doxycycline prevents a fatal infection but most hamsters develop a chronic leptospira renal infection. Daily treatment for 4 days or more, started at the time of infection or as late as 4 days after infection prevents death as well as chronic renal infection. Similarly, doxycycline

treatment of hamsters with a chronic renal infection is effective in eliminating leptospira from the kidney.

FUTURE OBJECTIVES :

1. Sera from hamsters immunized with different strains will be tested using an ELISA method for the diagnosis of leptospirosis. An antigen of broad cross reactivity for a many serovars will be sought to use in this test.

2. Hamsters will be used for testing water samples for leptospira infection. Hamsters of 40 grams in groups of at least 4 will be injected intraperitoneally to test a given sample.

4. Leptospirosis in the Non-human Primate Model : Chemoprophylaxis and Early Diagnosis of Infection

PROBLEMS : Leptospirosis is a common zoonotic disease found throughout the world. The clinical features in man range from an influenza-like illness to a more severe disease form manifested by continued fever with meningitic symptoms and signs. In some cases infection can lead to renal and hepatic failure, jaundice, and even death. Leptospirosis is frequently found in the tropical areas of the world and recent attention has focused on several outbreaks in soldiers training in jungle areas. Symptomatic treatment and antibiotic therapy are used in the acute illness. However, once symptoms are evident the beneficial effect of antibiotics is questionable. The relatively long recovery period, even with treatment, suggests that prevention is the practical approach in solving the problem of leptospirosis. It is difficult to prevent direct contact with leptospira contaminated water in a tropical environment, especially during military maneuvers. Immunization against specific serovars of leptospira can protect animals but immunization of man is not practical unless the serovar endemic to the area is identified or a vaccine with broad antiserovar activity is developed. Last year we reported that the experimental infection or monkeys with a local human isolate of the bataviae serovar produced a bacteremia of one to six days, infection of the CSF, and a bacteruria for up to four weeks. An antibody response was detected by microagglutination by one week and peak titers were reached by 3-4 weeks.

OBJECTIVES :

1. To characterize clinical leptospirosis in the non-human primate model.

2. To determine the efficacy of antibiotic treatment as a prophylaxis for the acute infection.

3. To determine if an ELISA method for detecting leptospira antibody or antigenemia is a useful means for obtaining rapid early diagnosis of leptospirosis.

PROGRESS : The inhibition of leptospira by different antibiotics was tested *in vitro*. Doxycycline was more effective than minicycline or tetracycline against three isolates of *L. bataviae*. Dicloxacillin had no effect at tested

concentrations up to 4 ug/ml. In a pilot experiment, the serum from 2 monkeys given doxycycline inhibited growth of leptospira *in vitro* when samples were taken as long as 72 hours after a single oral dose (5 mg/kg).

When eight monkeys were treated daily for 10 days with a single dose of doxycycline based on a 2-3 mg/kg equivalent dose for man, there was no bacteremia or the number of days of bacteremia was reduced. In addition, infection of the CSF and chronic renal infection occurred in placebo treated controls but was not seen in infected monkeys treated with doxycycline.

In two other groups of monkeys a single dose of doxycycline was given two hours prior to infection. In 6/8 doxycycline treated monkeys the number of days of bacteremia were less than in controls. In two monkeys the period of bacteremia was similar to infected placebo treated monkeys and one of these developed a chronic renal infection. In the eight placebo treated monkeys, six developed chronic renal infection and leptospira were cultured from the CSF of three. Leptospira were never isolated from the CSF of any doxycycline treated monkey. An ELISA method for detecting leptospira IgM was developed with Virology. To date the ELISA test has been used for monkeys and can detect IgM antibody to *L. bataviae*; however, in its present form this method is not as sensitive as the microagglutination method.

FUTURE OBJECTIVES :

1. Further develop and refine the ELISA to detect low antibody titers to leptospira.

2. Test sera from monkeys immunized with different serovar antigens by the ELISA method and find an antigen or combination of antigens that will detect serovar infections that are present in Thailand.

5. Effect of Antimalarial Drugs on Human Lymphocyte Response to Mitogenic Lectins

PROBLEM : Since immunosuppression is a characteristic of malaria infection the possibility that an antimalarial agent may itself compromise immune responsiveness becomes an important clinical consideration. A drug induced decrease in host immune capacity during malaria infection could result in a prolonged parasite clearance time and subsequent delayed recovery from the disease. Similarly, the compromise to the patient may result in increased susceptibility to intercurrent illness. There is also the concern for malaria endemic populations where suboptimal chemoprophylaxis may combine with the disease itself so as to compromise vaccine employment - especially a prospective malaria vaccine.

PROGRESS : Mitogenic lectin induced lymphocyte blast transformation provides an established assay for evaluation of cellular immune responsiveness. We have standardized an *in vitro* mitogenic lectin assay to assess whether selected antimalarial drugs suppress cellular immune responsiveness in human lymphocytes. Preliminary studies show that the new antimalarial drugs, mefloquine and halofantrine, suppress normal lymphocyte response to mitogenic lectins

(phytohemaglutinin, Concanavalin A and pokeweed mitogen). These drugs also suppress responsiveness of mononuclear cells isolated from malaria patients.

FUTURE OBJECTIVES : These studies represent *in vitro* conditions and may not accurately reflect the *in vivo* situation-especially when metabolites of a specific drug occur. A combination *in vitro/in vivo*, test system has been proposed as a more predictive measure for assessing the effect of antimalarial drugs on immune responsiveness.

6. Adenosine Deaminase in Malaria Infection : Effect of 2'-Deoxycoformycin *in vivo*

PROBLEM : Purine nucleotides are required by the rapidly proliferating malaria parasite for both energy metabolism and nucleic acid synthesis. The malaria parasite cannot synthesize purines *de novo* and depends for its intraerythrocytic (IE) growth and development on salvage of purine bases from the host RBC and extracellular environment. We have shown with *Plasmodium falciparum, in vitro*, that hypoxanthine is utilized as a purine base precursor for parasite synthesis of adenosine and guanosine nucleotides and that specific inhibition of these synthetic pathways leads to parasite destruction. Whether hypoxanthine is the malaria parasites preferred substrate *in vivo* is not known. An increase in adenosine deaminase (ADA) activity, however, is an obvious means for production of IE hypoxanthine. Increased availability of hypoxanthine would be a natural consequence of adenosine metabolism in the mature erythrocyte since this cell lacks of enzyme xanthine oxidase. Conversely, inhibition of ADA activity could act to deprive the rapidly growing IE malaria parasite of a readily accessible hypoxanthine pool for purine nucleotide synthesis.

PROGRESS : Adenosine deaminase from both human *P. falciparum* and monkey *P. knowlesi* has been characterized by an immunoassay. Host RBC ADA enzyme was precipitated with rabbit anti-human RBC ADA antibody bound to *S. aureus*. The non-immunoreactive parasite enzyme was recovered in the supernatant. The parasite enzyme has been characterized for its kinetic properties and response to inhibitors. Deoxycoformycin inhibited both parasite and host ADA whereas the competitive ADA inhibitor erythro-9- (2-hydroxyl-3-nonyl)-adenine (EHNA) was ineffective for the parasite enzyme. Deoxycoformycin administed to *P. knowlesi* infected rhesus monkeys produced a dramatic reduction in parasitemia implicating catabolism of adenosine as an important source of parasite hypoxanthine. Infection was not, however, eliminated by deoxycoformycin *in vivo* suggesting stage specificity of the agent or an alternative source of hypoxanthine once infection has progressed.

FUTURE OBJECTIVES : The project should be continued. The central role of hypoxanthine in malaria parasite purine metabolism provides a unique biochemical focus for antimalarial drug design. A series of new purine inhibitors have been developed and will be tested against *P. falciparum in vitro*. Further studies will be done on the question of stage-specificity for deoxycoformycin and the role of ADA in parasite development and proliferation.

7. *In vitro* Antimalarial Drug Sensitivity Testing

PROBLEM : In Thailand, *Plasmodium falciparum* is now resistant to conventional antimalarial drugs. This resistance varies from almost complete in the case of chloroquine and other 4-aminoquinolines and pyrimethamine/sulfadoxine to moderate but increasing for quinine. Quinine at high therapeutic dose continued to be effective when combined with tetracycline. Two new antimalarial drugs, mefloquine and halofantrine, have been introduced into Thailand and are now in various stages of evaluation and development. *In vitro* antimalarial drug sensitivity testing provides an objective means of quantifying dose-response characteristics for individual drugs and thus the identification of resistance patterns in Thailand.

PROGRESS : A radioisotope microdilution technique has been adapted to test antimalarial activity *in vitro* under field conditions. The technique was standardized in the central Bangkok laboratory. The technique is based on incorporation of (3 H) hypoxanthine by parasitized RBC in microculture. Inhibition of uptake of (3 H) hypoxanthine by the parasites serves has proven more sensitive and precise than traditional microscopic methods. It also permits large scale testing with fewer personnel. Data computation and records storage has been automated.

Antimalarial drugs are being tested in support of Phase III clinical evaluation studies and other field medical protocols. The drugs are : mefloquine, halofantrine, WR 180409, quinine, chloroquine, pyrimethamine and sulfadoxine. The data from *in vitro* testing has helped accomplish the following: (1) provide *in vitro/in vivo* correlation of antimalarial drug response; (2) establish base-line quantitative data (ID 50); (3) permit identification and collection of drug resistant malaria strains; (4) allow comparative testing of malaria strains from treatment failures when they occur; and, (5) support comparative epidemiological studies. Methods have been established for use of the radioisotope procedure in the field. Morphological field tests have been developed for testing of antifols. A cryobank has been established for preservation of reference strains. Additionally, work has begun on cloning of mefloquine resistant strains of *P. falciparum*.

FUTURE OBJECTIVES : Antimalarial drug resistance is an on-going problem. It is essential that antimalarial drug sensitivity testing be done on a continuing basis to support clinical trials and provide base-line epidemiological data on drug resistance in Thailand.

8. The Treatment of *Plasmodium falciparum* Malaria with Halofantrine, a Phenanthrenemethanol

PROBLEM : The rapid development of drug resistance in *P. falciparum* malaria necessitates the continuing process of new drug development for potent blood schizonticides which could replace mefloquine as an antimalarial treatment drug. Halofantrine (WR 171,669) is in Phase II testing.

In clinical Phase II studies performed in 27 nonimmune subjects infected with Vietnam Smith strain, *P. falciparum*, all 14 subjects who received

1000-1500 mg as split doses over a single day were cured. Only 3 RI recrudescences occurred in single doses of 1000 mg or 1500 mg. In addition the remaining 10 patients who received higher doses of the drug over a longer time period were all cured (1).

The important task was to determine field efficacy of this new antimalarial drug. Therefore 1500 mg split dose over one day was compared against mefloquine as single dose in a treatment trial of multi-drug resistant *P. falciparum* malaria on the Thai-Kampuchean border.

PROGRESS : From October 15, 1982 - October 15, 1983 at Ft. Taksin, Chantaburi in a population of Royal Thai Marines and volunteer soldiers naturally infected with *P. falciparum* malaria a double blind randomized treatment trial was conducted. These soldiers had parasite counts between 1000-100,000/mm3, and had no GI, renal or cerebral complications. They were dosed then observed as hospitalized patients in a non-malarious area for 21 days post treatment and were examined again at day 28 after returing to their units.

Fifty-nine patients were dosed with Halofantrine at 500 mg Q 6 hr x 3. 53/59 patients were cured (90%). There were 6 RI recrudescences. (1 day 12, 3 day 21, 1 day 26, and 1 day 28).

Thirty-eight patients were treated with mefloquine single dose. Twenty patients received a 1500 mg dose and 18 patients received a 1000 mg dose. 20/20 patients were cured at the 1500 mg dose (100%), and 17/18 patients were cured at the 1000 mg dose (94% cure rate). There was 1 RI recrudescence at day 28. The comparative parasite clearance times, (PCT), fever clearance times (FCT), initial parasite counts (IPC) and side effects are out lined below.

	Geometric Mean IPC/(per mm3)	Mean PCT	Mean FCT
Halo 500 mg q 6 hr x 3 n = 59	14,893	75 hr	54.6 hr
Mefloquine 1500 mg n = 20	12,745	64 hr	34.3 hr
Mefloquine 1000 mg n = 18	18,375	72 hr	53 hr

	Vomitting	Nausea	Diarrhea	Abdominal Pain	None
Halo 500 mg every 6 hr x 3 n = 59	20%	29%	22%	8.4%	42%
Mefloquine 1500 mg n = 20	20%	20%	45%	5%	40%
Mefloquine 1000 mg n = 18	28%	11%	39%	0	67%

FUTURE OBJECTIVES : In order to elucidate the degree of cross-resistance between mefloquine and halofantrine, a treatment trial should be performed with halofantrine patients receiving mefloquine after recrudescence and mefloquine failures receiving halofantrine treatment. Blood levels of halofantrine should be done on all future halofantrine patients at day 1, 3 and 5.

A pharmacokinetic study of halofantrine in patients infected with *P. falciparum* would develop guidelines to judge the adequacy of blood level in malaria patients treated with halofantrine.

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9. The Comparison of Mefloquine and Doxycycline as Clinical Prophylactic Agents for Falciparum and Vivax Malaria in Royal Thai Army Troops Assigned to the Thai-Kampuchean Border

PROBLEM : Antimalarial prophylaxis in South East Asia has been of great concern with the emergence of chloroquine resistance in the 60's and 70's and Fansidar resistance in the 80's. The last meaningful prophylactic study was performed by AFRIMS in Prachinburi Province in 1976 comparing Fansidar and Mefloquine (1). It revealed a greater than 95% protection rate for both Fansidar and Mefloquine given either weekly or every two weeks using a double dose against *P. falciparum*. Fansidar had a 91%-93% protection rate against *P. vivax*. Current CDC and WHO recommendation for Thailand are Chloroquine plus Fansidar administered on a weekly basis.

We examined the prophylactic efficacy of three antimalarial drugs in a highly endemic area.

PROGRESS : The duration of this study was 14 weeks from 5 July 1983 - 14 October 1983. Three hundred and twenty two patients were enrolled from four sites along the Kampuchean border (Khao Tangoc, Khao Sarapee, Khao Din, and Khun Phol). They were randomly assigned to one of three prophylactic groups- mefloquine 250 mg weekly, chloroquine 300 mg base plus fansidar (500 mg sulfadoxine with 25 mg pyrimethamine) or doxycycline 200 mg 2 x/week.

Forty-three patients have been positive to date out of 322 patients. None were in the mefloquine group. Attack rates for each drug group will be calculated and the significant difference between groups will be noted.

FUTURE OBJECTIVES :

1. Mefloquine prophylaxis on a weekly basis is 100% protective even in this area of multi-drug resistant malaria.

2. Doxycycline should be evaluated as a 100 mg/day prophylactic dose.

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10. The Pharmacokinetics of Intravenous Quinine in Patients with Naturally Acquired Falciparum Malaria

PROBLEM : Quinine continues to hold the distinction of being the only parenteral treatment drug for chloroquine resistant *P. falciparum*. Intravenous administration of quinine is essential for treating critically ill patients. The success of quinine treatment depends on achieving adequate blood levels of the drug. There are known to be large interindividual variations in plasma quinine levels and we hope to identify the major factors accounting for such variability in quinine disposition in patients undergoing intravenous therapy.

PROGRESS : One IV infusion of quinine HCL 650 mg, in 500 ml of normal saline was administered over two hours and serial blood and urine samples were collected over a 26 hour period. The final five of nineteen patients completed this study between Oct. 1982 - Oct. 1983. There was one RII resistant patient to the followup therapy of quinine 650 mg tid x 3 days with tetracycline 500 mg-250 mg-250 mg x 7 days.

The study was concluded and awaits High Performance Liquid Chromatography analysis of the serum and urine quinine levels and the synthesis of a pharmacokinetic curve.

The arithmetic mean initial parasite count of the group was 31,284 per mm3. The mean parasite clearance time was 97.2 hours and the mean fever clearance time was 87 hours, following a treatment program of quinine 650 mg tid x 3 days and tetracycline 500 mg-250 mg-250 mg tid x 7 days.

FUTURE OBJECTIVES : Pending data analysis.

11. The Comparison of Chloroquine, Chloroquine-Fansidar and Mefloquine as Clinical Prophylactic Agents for *P. falciparum* and *P. vivax* Malaria in Thai Gem Miners Along the Thai-Kampuchean Border

PROBLEM : The goal of this study was to assess if chloroquine plus fansidar has a prophylactic efficacy superior to chloroquine alone, and to compare both to the best available antimalarial, mefloquine.

PROGRESS : This study began on July 21, 1982 in Borai, Thailand. As of 15 October 1983, 199 subjects had been enrolled in this prophylactic trial. Patients have been randomly assigned to one of three drug groups (1) chloroquine (300 mg base/weekly) plus fansidar (1000 mg sulfadoxine and 50 mg of pyrimethamine) every 2 weeks, (2) chloroquine 300 mg base weekly or (3) mefloquine 500 mg

every two weeks. Medications are administered in a double-blinded fashion. Forty-five patients were positive to date : 5/45 on week 1, 24/25 on week 2, 3/45 on week 3, 5/45 on week 4, 1/45 on week 5, 2/45 on week 6, 2/45 on week 8, 3/45 on week 10, all with *P. falciparum*. Fifty-nine patients were discharged from the study due to poor compliance with followup visits. Seventy-seven patients continue in the study to date.

FUTURE OBJECTIVES : Our goal is to follow 450 patients to either ppsitivity or to complete 14 week follow up.

12. Comparative Bioavailability and Renal Clearance of the Combination of Quinine and Tetracycline Given Simultaneously or Sequentially

PROBLEM : To evaluate the bioavailability of tetracycline when administered sequentially or simultaneously with quinine and to assess the effect quinine has on the renal clearance of tetracycline has on serum levels of quinine.

PROGRESS : A total of 25 patients comprised this study population at Phrabuddhabhat Hospital in central Thailand. Eleven of twenty-five patients were treated between October 1982 and October 1983. The clinical portion of the study is now complete and awaits biochemical and pharmacokinetic analysis at WRAIR.

The mean age of both sequential (Group I) and simultaneous (Group II) Q-T was 28 years old. The mean weight for group I was 53 kg and for Group II was 57 kg.

Side effects were not significantly different in each group.

	Mean Parasite Clearance Time	Fever Clearance Time	Mean Initial Parasite Count
Quinine 650 mg x 3 days followed by Tetracycline 1 gm per day x 7 days (Group I) n = 12	92 hr	66 hr	21,780
Quinine 650 mg x 3 days together with Tetracycline 1 mg per day x 7 days (Group II) n = 13	88 hr	59 hr	18,864

FUTURE OBJECTIVES : Pending pharmacokinetic analysis. Tinnitus was universal, perhaps indicative of adequate blood levels, while blurred vision as a sign of drug toxicity was seen in three patients (two Group I and one Group II).

13. The Treatment of *P. falciparum* Malaria with a Combination of Quinine and Tetracycline

PROBLEM : To determine the efficacy of the combination of quinine and

Tetracycline in various treatment regimens and to compare them to mefloquine for efficacy and severity of side effects. All patients were randomized and treated with one of four drug regimens : mefloquine 1500 mg single dose, quinine 650 mg every 8 hr x 6 days and tetracycline 500 mg every 8 hr x 6 days (Q6 T6), quinine 650 mg every 8 hr x 3 days and tetracycline 500 mg every 8 hr x 7 days (TQ3 T7) or quinine 650 mg loading dose followed by quinine 325 mg every 6 hr x 3 days plus tetracycline 250 mg every 6 hr x 7 days (Q3 T7). The patients were examined daily for symptoms or physical findings and hospitalized for 21 days for a 28 day follow up period in a non-malarious area.

PROGRESS :	Arithmetic Mean Initial Parasite Count	Mean Parasite Clearance Time	Mean Fever Clearance Time	Cure Rate	Recrudescences
Mefloquine n = 26 19/26 between Oct 82-Oct 83	23,033 per mm3	65.2 hr	40.4 hr	92% (24/26)	1 RI day 21 1 RII not cleared in 14 days
Q6 T6 n = 42 18/42 between Oct 82-Oct 83	23,403 per mm3	86.5 hr	58.5 hr	100% (42/42)	4 *P. vivax* relapses 1 day 21 2 day 29 1 day 28
Q3 T7 High Dose n = 20 18/20 between Oct 82-Oct 83	29,933 per mm3	92.5 hr	64 hr	90% (17/19)	2 RI day 28 1 lost to followup day 21
Q3 T7 Low Dose n = 15 15/15 between Oct 82-Oct 83	21,253 per mm3	106 hr	57 hr	93% (14/15)	1 RI day 28 2 *P. vivax* 1 day 27 1 day 28

FUTURE OBJECTIVES : The study will be continued until 40 patients are in each group. To date mefloquine has comparable efficacy with Q3 T7 (high or low dose).

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